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Effects of blue, orange and white lights on growth, chlorophyll fluorescence, and phycocyanin production of *Arthrospira platensis* cultures.

[1]G. Chini Zittelli, [1]G. Mugnai, [2]M. Milia, [1]B Cicchi, [3,4]A.M. Silva Benavides, [2]A. Angioni, [5]P. Addis, [1,4] G. Torzillo *

*giuseppe.torzillo@cnr.it (corresponding author)

[1]CNR-Istituto per la Bioeconomia, Via Madonna del Piano 10, Sesto Fiorentino, I-50019 Florence, Italy

[2] Department of Life and Environmental Science, Food Toxicology Unit, University of Cagliari, University campus of Monserrato, SS 554, 09042 Cagliari, Italy

[3] Escuela de Biologia, Universidad de Costa Rica, San Pedro de Montes de Oca, San Jose, Costa Rica

[4]CIMAR – Centro de Investigación en Ciencias del Mar y Limnología, Universidad de Costa Rica, San Pedro de Montes de Oca, San Jose, Costa Rica

[5]Department of Life and Environmental Science, Biology Section, University of Cagliari, via Tommaso Fiorelli 2, 09126 Cagliari, Italy

Abstract

The aim of this study was to evaluate the effects of different light colors on growth, pigment composition, and photosynthetic performance of *Arthrospira platensis*. Results showed that under orange light the biomass productivity increased due to the capability of *A. platensis* to fully absorb this portion of the light spectrum. Under blue light, phycocyanin increased continuously up to 13.2% ± 1.96 of dry weight at day 5, while under orange and white lights the phycocyanin content resulted lower, 7.1 ± 0.39 and 6.7% ± 1.58 of dry weight, respectively. Chlorophyll fluorescence measurements showed the maximum electron transport rate ($rETR_{max}$) in cells grown under orange light. The results of this study indicated that the orange light increased both growth and phycocyanin productivities, while blue light increased mostly the phycocyanin content, while biomass productivity was much lower. Further increase of phycocyanin content was observed shifting the light illuminating the cultures from orange to blue, attaining a raise in phycocyanin content from 8.6% to 12.5% of dry weight within 48 hours from the start of the illumination with blue light. Within the same period of time no growth was observed indicating that the synthesis of phycocyanin can be decoupled from growth. This study provides useful physiological information regarding the effects of different light spectra on growth, phycocyanin, and photosynthetic performance, as a prerequisite to optimize the production of high value pigments from cultures of *A. platensis.*

Keywords: Cyanobacteria; Chlorophyll fluorescence; Light spectra, Phycocyanin; *Arthrospira platensis*

1. Introduction

The photosynthetic apparatus of cyanobacteria contains chlorophyll *a* and the accessory phycobiliprotein pigments, phycocyanins (PC), allophycocyanins (AP) and including phycoerythrins (PE) [1]. They consist of an apo-protein and one or more chromophores, also known as bilins. Phycobiliproteins constitute specialised light-harvesting antenna pigments, forming larger protein complexes called phycobilisomes (PBSs) composed of an allophycocyanin core and staked rods of phycocyanin often in combination with phycoerythrin. PBSs transfer their energy to photosystem II (PSII) thus balancing the higher light absorbing capacity of PSI due to its higher chlorophyll content, and the higher PSI/PSII ratio which is usually much higher than 1:1 (between 5:1 and 2:1) typical for microalgae and higher plants. Moreover, there are evidences that cyanobacteria can re-equilibrate excitation energy by moving PBSs between PSII and PSI in a process called state-transition within a time scale of seconds to minutes [2].

These water-soluble bright blue (PC and AP) and red PE pigments, have commercial applications as antioxidant, anti-inflammatory, neuroprotective, anti-cancer and immunomodulatory [3,4]. PC is obtained from *A. platensis* has been approved as a food colouring by the FDA in August 2013. According to a report by Future Market Insights it is estimated a global total market of about US$ 224 million by 2028 [5]. The largest consumers of phycocyanin are the Western European countries (33%), mostly used in food industry (80%) [5]. However, the market price of phycobiliproteins is strongly depended on their purity degree. For example, highly purified molecular markers labeled with antibodies of other fluorescent molecules can reach as much as US$ 1500 per 1 mg [6].

However, the commercial use of PC has been limited by the high cost of production, large-scale extraction and particularly purification, which is still problematic and expensive [7]. The PC content can be manipulated by both light intensities supplied to the culture, and by modulating the light spectrum. In general, light limitation, achieved either with a low incident

light intensity or by increasing the biomass concentration of cultures, enhances the chlorophyll and phycocyanin contents, while when microalgae are growing fast, that is under light saturation, a large amount of nitrogen is required and phycocyanin could be consumed as an alternative source of nitrogen to sustain biomass growth [8,9]. High oxygen concentration in the cultures, especially in those grown in closed photobioreactors, causes a reduction of phycocyanin and protein contents [10]. Nowadays, artificial light is often used for the production of high value-added compounds such as phycobiliproteins, carotenoids and astaxanthin.

Artificial lighting can be used to manipulate the final biomass for specific applications. To design an artificial lighting system for microalgal growth, the electronic and photosynthetic efficiency under different light sources must be considered. Fluorescent lamps and light emitting diodes (LEDs) are two different types of light sources used for the microalgal cultivation. During the late 20th century to early 21st century, the first studies on the effects of light source on the growth of *A. platensis* were carried out using cool light fluorescent lamps covered with colored cellophane papers [11–14]. These studies were aimed at investigating the production of phycocyanin [11,13,15], and biomass [12,14] by *A. platensis* under different light spectra. With the introduction of LEDs several studies were conducted using this light sources [14,16–20]. However, fluorescence tubes have the advantage to deliver light irradiance according to a 360° angle and are usually characterized by a much larger emission bands compared to LEDs. On the other hand, due to the small size, light weight, quick lighting durable and efficient energy conversion and longer operating life, the use of LEDs has become predominant. However, although LEDs have several advantages that have promoted their diffusion as the light source for the purpose of microalgae growth, particularly when microalgae are grown under flashing light, recently a few studies on the growth of *A. platensis* were performed using fluorescent lamps [21].

In order to achieve high yields of biomass and high value products, the light source color is an important issue that should also be taken into account. Photosynthesis in *A. platensis*, similar to other cyanobacteria, is performed through the light harvesting complex, constituted of carotenoids, phycobilisomes and chlorophyll. The absorption maximum and fluorescence emission maximum ($\lambda_{AX}\backslash\lambda_{EX}$) are 498/576 nm for phycoerythrin, 620/642 nm for phycocyanin and 651/662 nm for allophycocyanin. PBSs capture sections of the solar spectrum not used by chlorophyll molecules and very efficiently transmit energy to the photosystems where charge separation takes place [22]. Different light spectra may represent an abiotic stress for *A. platensis*, affecting the photosynthetic efficiency and the oxygen uptake. Although several studies have been carried out to evaluate the effects of different light colors on the cell growth and phycocyanin production of *A. platensis*, a critical investigation on the physiology of growth and on the total output of phycocyanin by cultures of *A. platensis* remains still controversial. Therefore, the aim of this study was to compare the photosynthetic performance, biomass growth, and phycocyanin output of *A. platensis* cultures grown attained under three different light colors (blue, orange and white). Chlorophyll fluorescence quenching was used to evaluate the photosynthesis performance of the cultures.

2. Material and Methods

2.1. Strain and culture conditions

Arthrospira platensis, strain M2, from the culture collection of National Research Council (CNR), Institute of Bioeconomy (IBE), at Sesto Fiorentino (Italy) was used. The culture was grown in Zarrouk's medium [23] in a batch mode. Growth experiments were carried out in vertical glass columns (5 cm light path, 400 mL working volume) placed in a water bath at

30 ± 1 °C and bubbled with a mixture of air/CO_2 (97/3, v/v) at a continuous flow rate of 5 L min^{-1}.

Cultures were grown under three light colors: white (peak 437 nm, and 630 nm), blue (peak 450 nm), and orange (615 nm) provided by means of fluorescent tubes mounted on two parabolic stainless steel reflective supports providing uniform light distribution on the surface of the cultures. Incident light intensity can be adjusted by changing the distance of the light source from the culture vessel (Fig. 1).

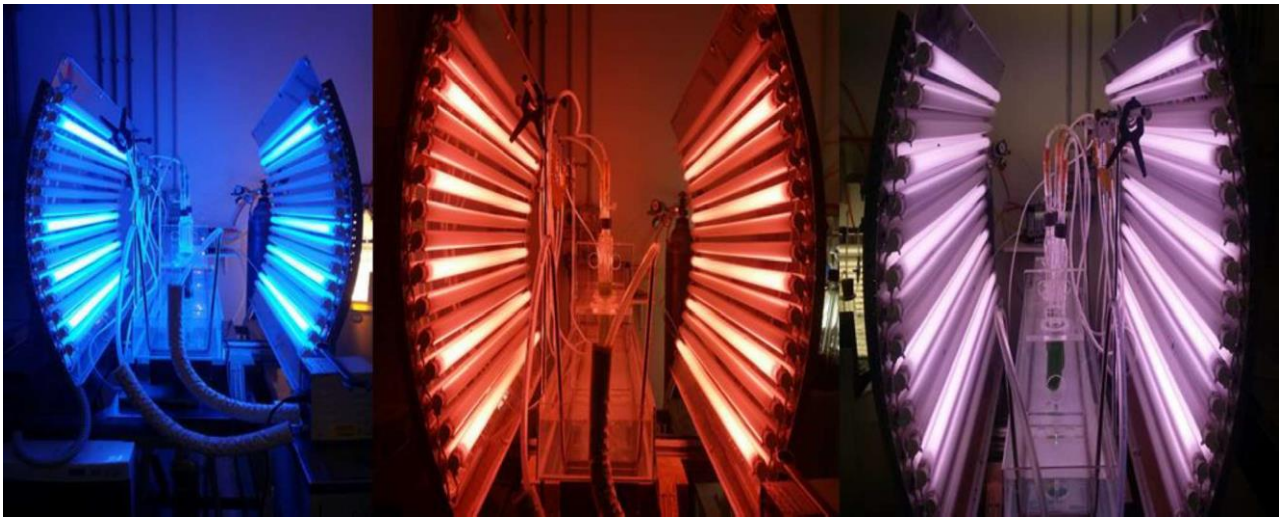


Fig. 1. Cultivation apparatus used for cultivating *A. platensis* under three light sources (blue, orange and white lights).

The culture was acclimated to white, blue and orange lights for 3 days under continuous lighting before the start of measurements. At the starting point (0 h) the cultures were diluted to the set biomass concentration of 230 ± 30 mgL^{-1}. The culture temperature was maintained constant at 30 °C, by partially immersing the columns in a thermostated water bath. Mixing of the strain was performed by air bubbling. Culture pH was maintained at 9.4 by a mixture of air/CO_2 (97/3v/v). Photon flux density (PFD) of 90 µmol m^{-2} s^{-1} was supplied from both sides of the culture vessel (Fig. 1). Spectral UV measurements in this study were carried out using a double monochromator spectroradiometer (model SR9910-PC, Macam

Photometrics Ltd, Livingstone, Scotland) with a 100 mm focal length, a spectral range of 280-800 nm, a wavelength accuracy of 0.5 nm, equipped with a diffuser connected by a 1.8 m long optical fiber to the input slit of the monochromator (Fig. 2).

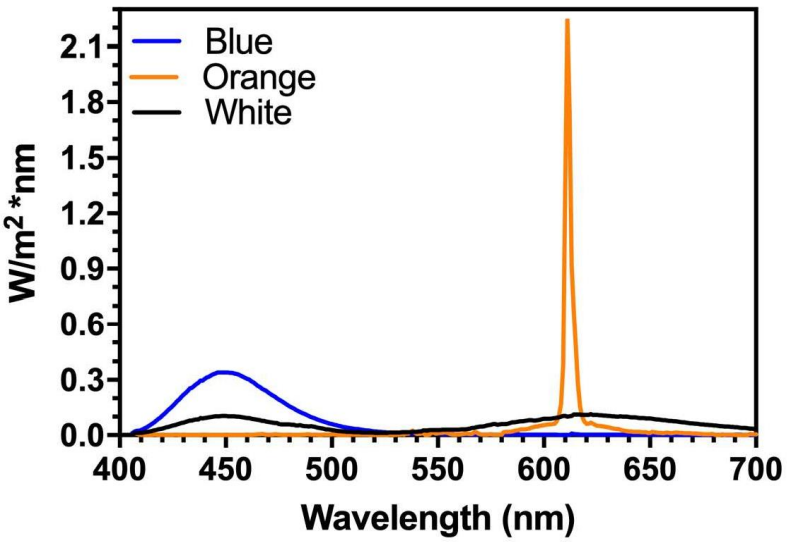


Fig. 2. Light emission spectra of the three light sources (blue, orange and white) obtained using a double monochromator spectroradiometer. Data represent the average of 3 replicates.

2.2. Analytical procedures

Determination of dry biomass weight (DW) was carried out in triplicate using 5 mL culture samples filtered with pre-weighted 47 mm diameter glass microfiber filter membranes (Whatman GF/F, Maidstone, England); quickly washed twice with deionized water, oven dried at 105 °C for 3 h and weighed on an analytical balance. Chlorophyll and carotenoid contents were determined according to Lichtenthaler, using pure methanol as a solvent [24]. Cyanobacterial biomass was centrifuged at 4000×*g* for 5 min and the pigment concentration was determined spectrophotometrically with a Varian Cary50 UV-visible spectrophotometer (Varian, Mulgrave, Australia).

2.3. Phycocyanin, allophycocyanin and protein measurements

For phycocyanin and allophycocyanin extraction, samples were centrifugated at 4000×*g* for 5 min, then the biomass was separated from the supernatant and mixed with 5 mL of 1% calcium chloride ($CaCl_2$) for 2 min. These solutions were subjected to three cycles of freezing and thawing, then left incubating at 4 °C overnight. Next, the samples were centrifuged at 9000×*g* for 10 min and then the absorbance of the supernatant was measured at 620 nm (phycocyanin) and 652 nm (allophycocyanin) with Varian Cary50 UV-visible spectrophotometer (Varian, Mulgrave, Australia). The phycocyanin and allophycocyanin concentrations were calculated using the following equations [25]:

$$\text{Phycocyanin (mg/L)} = (A_{620} - 0.474 \times A_{652}) / 5.34)$$

$$\text{Allophycocyanin (mg/L)} = (A_{652} - 0.208) \times A_{620} / 5.09)$$

where A_{620} and A_{650} are the optical density at 620 at 650 nm, for phycocyanin and allophycocyanin, respectively. Protein determination was assessed according to Lowry et al. [26].

2.4. Measurement of specific chlorophyll optical absorption cross-section

The average specific chlorophyll optical absorption cross-section (a^*) of the cells (m^2 mg^{-1} Chl *a*) was measured from *in vivo* absorption spectra (400 to 750 nm range) according to Kromkamp et al., (1993) [27] using a double-beam spectrophotometer (Varian Cary50 UV-visible). To minimize the effect of the light scattering effect from the cell surface, the sample cuvette was placed close to the detector window.

2.5. Fluorescence measurements

Chlorophyll fluorescence measurements were carried out using a pulse-amplitude-modulation fluorometer (PAM-2100, H. Walz, Germany) operated with PamWin (version 2.00f) PC software. The ratio between variable and maximum fluorescence (F_v/F_m), was used to assess the maximum photochemical quantum yield of photosystem II (PSII). Prior to fluorescence measurements, samples were incubated in the dark for 10 min to remove any energy-dependent quenching. Then, just before the F_m determination, one far-red light (above 700 nm) pulse with a duration of 10 sec (10 W m^{-2}), supplied by the PAM-2100, was applied to oxidize the PQ pool. In cyanobacteria, the effective ETR according to Genty et al. (1989) [28] (ETR= PFD × Yield × 0.5 × 0.84) is not used since it cannot be accurately determined because in cyanobacteria the ratio between PSI/PSII is highly variable and the absorption coefficient is very different from 0.84 assumed for leaves. Because of these limitations, we calculated a relative rETR, by neglecting the PSI/PSII ratio, and multiplying the result by the optical cross-section (a*), that is, rETR = PFD × Yield × a*. This calculation represents a better approximation of the effective ETR as compared to the values given by the PAM-2100 software which was designed for higher plant.

2.6. Statistical analysis

All the analysis was conducted in experimental triplicates (N=3); a minimum number of three instrumental replicates was always conducted for each measurement (n=3). Data were analyzed using one-way or two-way analysis of variance (ANOVA) at a significance level of $p < 0.05$, after homogeneity test. To correlate parameters, linear regression analyses were performed, reporting r^2 and P values in each case. Student's t-test was used between the variables groups and significant levels were set at $p < 0.05$ levels. Statistical analysis was performed using GraphPad Prism version 6.00 (GraphPad software, California, USA).

3. Results

3.1. Absorption cross section

The cyanobacterium *A. platensis* shows absorption peaks in the blue and red part of the spectrum (440 and 680 nm), due to chlorophyll a (Chl *a*), and in the orange part (620 nm) due to phycocyanin (Fig. 3). As can be seen, different light colors at which cultures were exposed, produced relevant differences in the absorption spectrum. The ratio between the phycocyanin peak at 620 nm and chlorophyll at 680 nm was higher under blue light than under the orange and white lights, indicating that the phycocyanin content increased relatively to chlorophyll when cells were exposed to blue light. Moreover, the absorption shoulder at 450-500 nm, indicative of carotenoids, increased under white light and in blue light (Fig. 3).

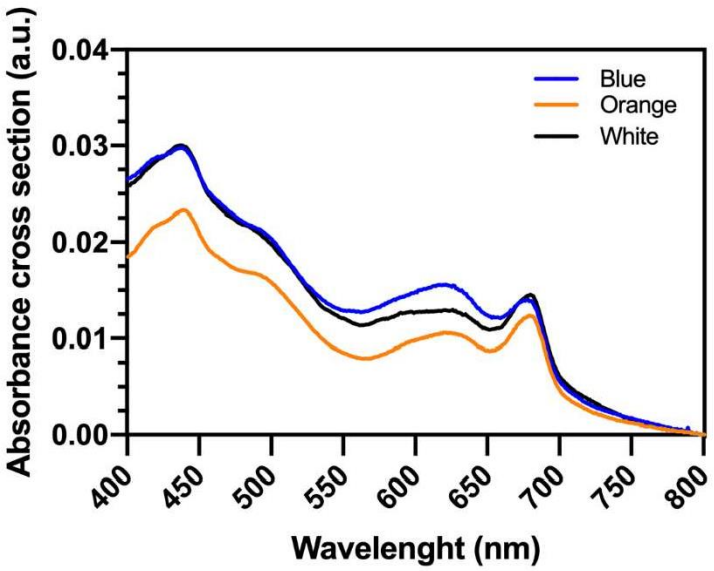


Fig. 3. In-vivo light absorption spectra of *A. platensis* cells grown under white, orange and blue lights**.** Results, represent the mean of three independent biological replicates including all days of culture.

The averaged values of cross section for the different light colors were (m^2/mg Chl *a),* 0.015156 ±0.0023 (white light), 0.011635 ± 0.001255 (orange), and 0.016638 ± 0.002287 (blue). The largest cross section was therefore observed under blue light due to the higher contribution of phycocyanin, while the lowest was recorded with cells exposed to orange light most likely due to the low chlorophyll content and by a lower carotenoid content which may have reduced absorption (Fig. 3).

3.2. Growth

The growth of *A. platensis* evaluated both in terms of chlorophyll *a* and dry weight increase attained under different light treatments is shown in Fig. 4a and 4b.

The chlorophyll *a* content of the cultures after 48 h were considerable different; under the white light a significantly higher chlorophyll *a* accumulation compared to the other light exposures was observed; under the white light chlorophyll was about 3 times greater than in cultures grown under blue light (Fig. 4a).

Biomass dry weight was significantly higher in cultures exposed to white and orange lights compared to blue light. DW at the end of the experiment (day 5) was about 3 times (orange light) and 2.8 (white light) times greater than under blue light conditions (Fig. 4b). Moreover, a strong positive correlation was found between biomass concentrations and chlorophyll contents (r^2 = 0.97, 0.98 and 0.79; $P < 0.1$ with blue, white and orange light respectively). Although there were not significant differences between the three light conditions during the first 24h, the effect of different light exposures was relevant also in the chlorophyll-to-dry weight ratio (Fig. 4c). The chlorophyll content (% DW) under the blue light was similar to that obtained under white light, excepts after 48 h where it was the highest, whereas under orange light, showed the lowest values from day 2 onward.

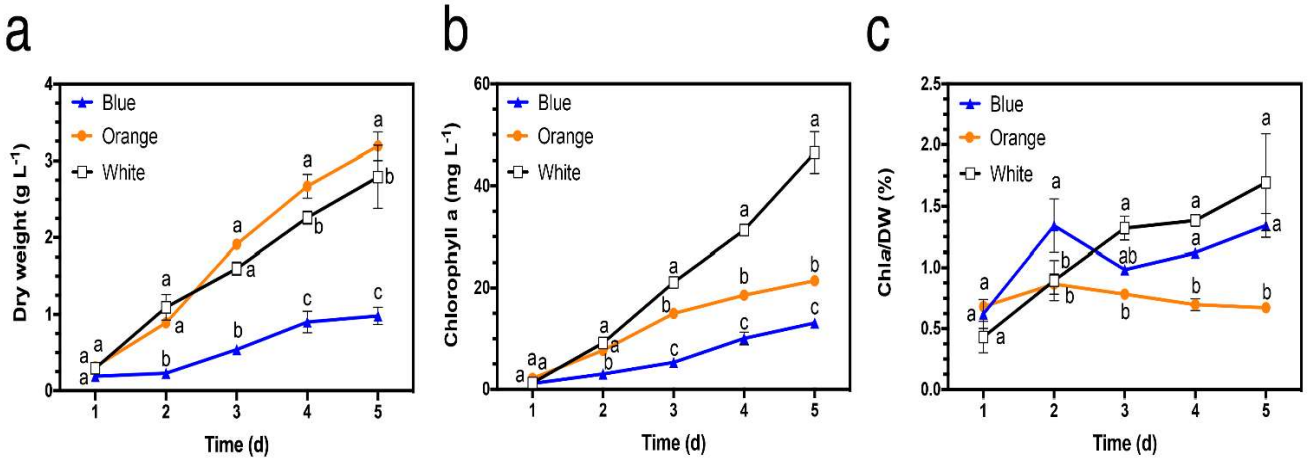


Fig. 4. Chlorophyll *a* (a) dry weight (b) and Chl:DW ratio (c) during the growth of *A. platensis* under different light color treatments (white, orange and blue lights). Bars indicate the standard deviation of 3 biological replicates, while different letters indicate significant difference ($p < 0.05$) between cultures exposed to different light spectra (white, orange, blue) within the same cultivation day.

3.3. Protein, phycocyanin and allophycocyanin contents

Changes in protein, phycocyanin and allophycocyanin contents were monitored during the experiment (Fig. 5 a - e). In cultures grown under blue and white lights, the proteins content was significantly higher compared to the orange light conditions, whereas there were not significant differences between the white-light and the blue light exposure. Under blue and white lights, it reached 75% of DW, while under orange light it was much lower, about 60% DW (Fig. 5a).

Blue light provided the lowest final phycocyanin concentration after 5 days (128.63 mg L^{-1}) but it indeed resulted the highest in terms of percentage of DW reaching a value of 13.23% (Fig. 5b and 5d). Instead, the orange and white light treatments did not show significant variations after 5 days of cultivation with a maximum value of 7.1 % of DW and 6.7 % of DW respectively. The same trend was also observed in the allophycocyanin content, where the cultures exposed to the blue light showed the lowest allophycocyanin production, reaching

a maximum of 32.72 mg L^{-1} after 5 days corresponding to 3.37% of DW (Fig. 5c and 5e). Furthermore, the allophycocyanin contents of the cultures exposed to the orange and white light increased during the experiment period with a maximum value of 76.20 mg L^{-1} (2.39% of DW) and 69.88 mg L^{-1} (2.56% of DW) respectively.

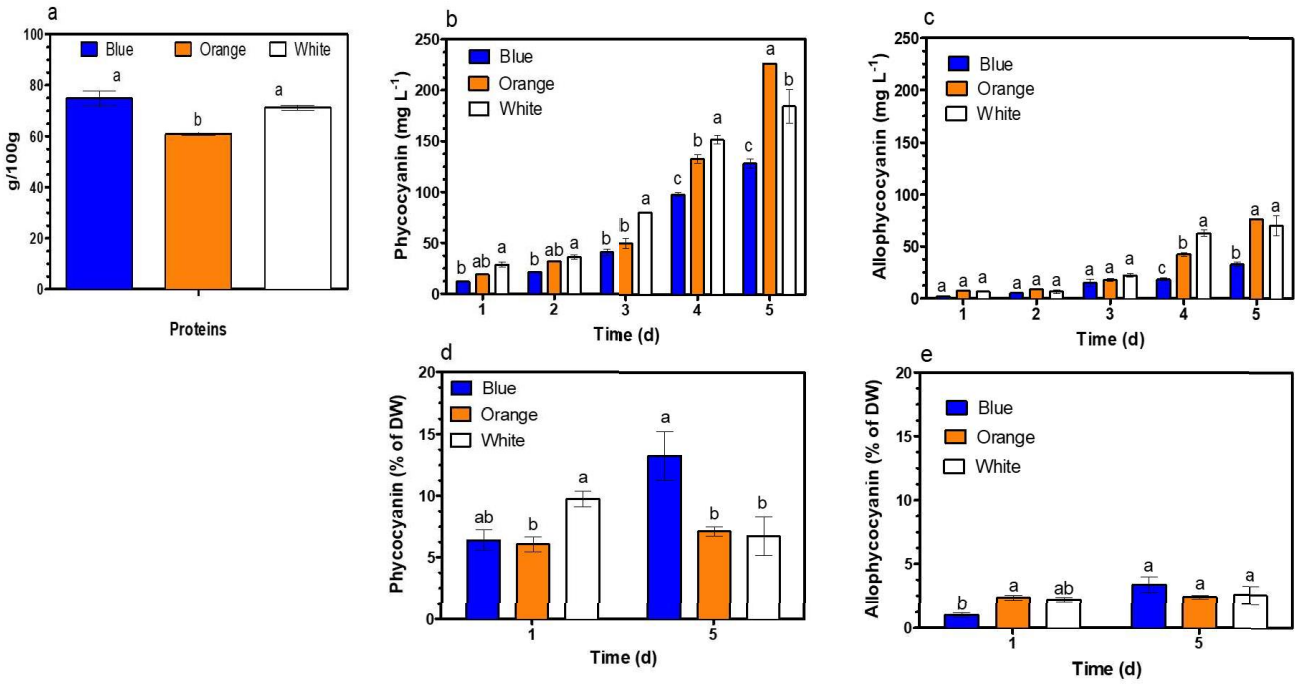


Fig. 5. Proteins content g/100g of dry weight at the end of the *A. platensis* cells growth (a). Phycocyanin (b) and allophycocyanin (c) content expressed in terms of mg L^{-1} and of % of DW (c-d) respectively, during the growth of *A. platensis* under different light color treatments (white, orange and blue lights). Bars indicate the standard deviation of 3 biological replicates, and different letters indicate significant difference ($p<0.05$) among light conditions within the same cultivation time (days).

3.4. Carotenoids

Analysis of the carotenoids showed similar profiles at 24h from the start, as a higher content was observed in the cultures exposed to orange and white lights during all the duration of experiment, reaching a maximum concentration of 8.01 mg L^{-1} (0.20% of DW) and 6.5 mg L^{-1} (0.29% of DW) respectively, after 5 days. Although the blue light exhibited the lowest

accumulation of carotenoids in terms of mg L^{-1} (Fig. 6a), it presented the highest ratio with respect to dry weight (0.36% of DW) after 5 days (Fig. 6b).

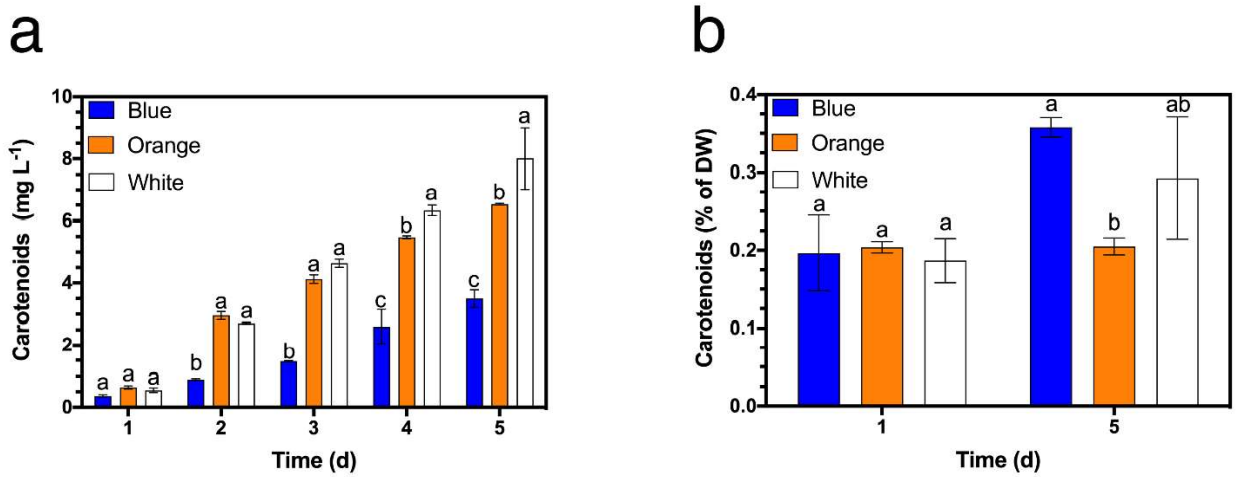


Fig. 6. Carotenoid content in mg L^{-1} (a) and in terms of % of DW (b) in *A. platensis* cultures grown under different light colors (blue, orange and white lights). Bars indicate the standard deviation of 3 biological replicates, while different letters indicate significant difference ($p < 0.05$) within the same cultivation time (days).

3.5. Chlorophyll fluorescence

Chlorophyll fluorescence measurements performed on *A. platensis cells* grown under different light treatments are presented in Fig. 7 a-b. The F_v/F_m ratio was generally higher under blue light exposure. It ranged within 0.56 to 0.65 under blue light, within 0.36 to 0.48 under orange light and within 0.34 to 0.55 under white light (Fig. 7a). The maximum electron transport rate of PSII ($rETR_{max}$) under saturating light were significantly greater under orange light (Fig. 7b), while no clear pattern was found between blue and white light.

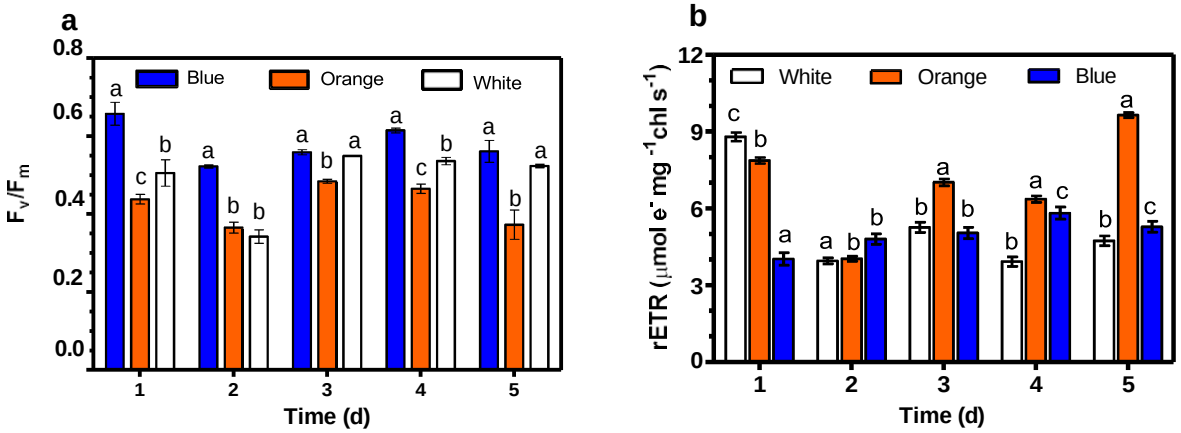


Fig. 7. Changes of the maximum photochemical quantum yield of PSII (F_v/F_m) (a), the relative maximum electron transport rate ($rETR_{max}$) (b) measured in *A. platensis* cultures grown under different light color (blue, orange and white). Bars indicate the standard deviation of 3 biological replicates, while different letters indicate significant difference ($p < 0.05$) within the same cultivation period.

Shift of culture from orange to blue light to enhance PC content

As blue light resulted the most effective radiation to increase the phycocyanin content, while the orange one the most effective in promoting growth, we reasoned about the possibility to further enhance the output of phycocyanin by adopting a two-step protocol. For this purpose, cultures of *A. platensis* were first grown under orange light until their stationary phase and, thereafter, they were shifted to blue light. The results of this experiment are shown in Fig. 8. High density cultures (about 5 g/L DW) with stable phycocyanin content close to 9% of dry weight during the last 3 days under orange light, once shifted to blue light showed a temporaneous decrease in their PC content. This drop was the result of a lack of nitrogen in the medium caused by the high biomass concentration reached by of the culture (close to 5 g/L) which exhausted the nitrogen. Indeed, once nitrate was added, a constant raise in the PC content was observed. Although it took 4 days to restore amount of PC found at the

start of the blue light phase (Fig. 8), the PC content increased consistently thereafter up to 12.5%, mainly due to PC synthesis and marginally by the drop in the DW, whose decline (about 15% reduction) caused a parallel increase in the concentration of PC in the cells. This experiment demonstrated that even dense cultures, at their stationary phase (no apparent growth) were still able to enhance their PC content under blue light provided that the nitrogen is not limiting.

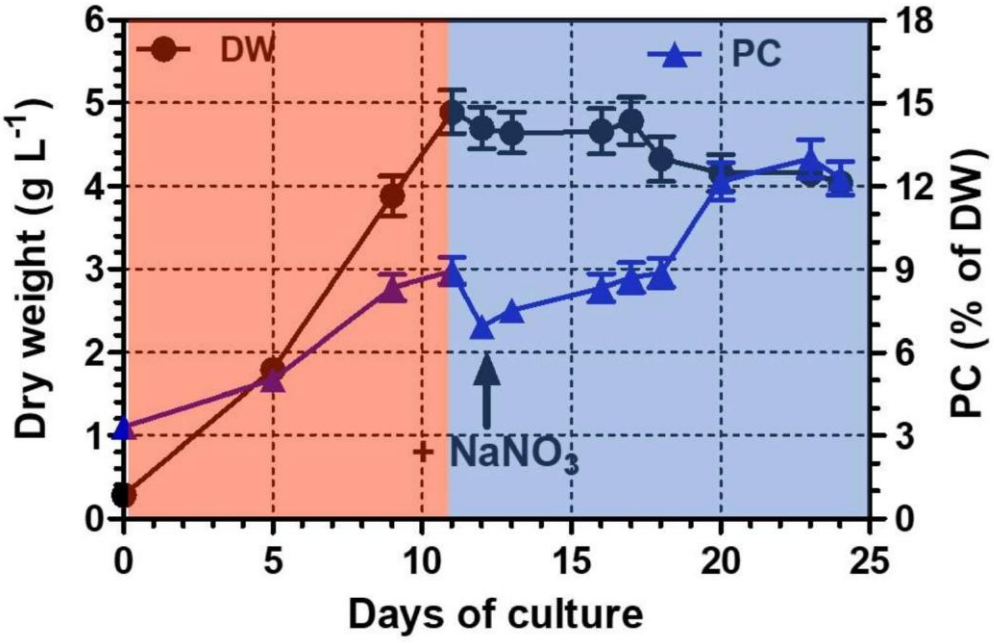


Fig. 8. Time course of growth (DW) and phycocyanin accumulation in culture of *A. platensis* grown under orange light for 5 days and thereafter shifted to blue light. The arrow indicates the day 2.5 g/L $NaNO_3$ was added to restore the initial nitrogen content of Zarrouk's medium. Data are presented as the mean ± SD of 3 biological replicates.

We wished to demonstrate that these culture, although at their stationary phase, were still photosynthetically competent. For this purpose, we measured the rETR in a culture sample taken from the blue light exposed cultures. As can be seen in Fig. 9 (a), the cultures retained about 60-70% of the rETR max values reported in Fig. 7b indicating that the culture during the accumulation of PC were active although their dry weight didn't change or it started to

decline. The sizeable reduction in the $rETR_{max}$ can be explained by the acclimation of the photosynthetic apparatus to low light conditions, as a result of the high density of the culture (about 5 g/l) during the shift to blue light. Further demonstration of the culture vitality came from the increase of the pH during the exposure to blue light (not shown). This experiment made clear that accumulation of PC can be decupled from the growth of the culture (increase in dry weight).

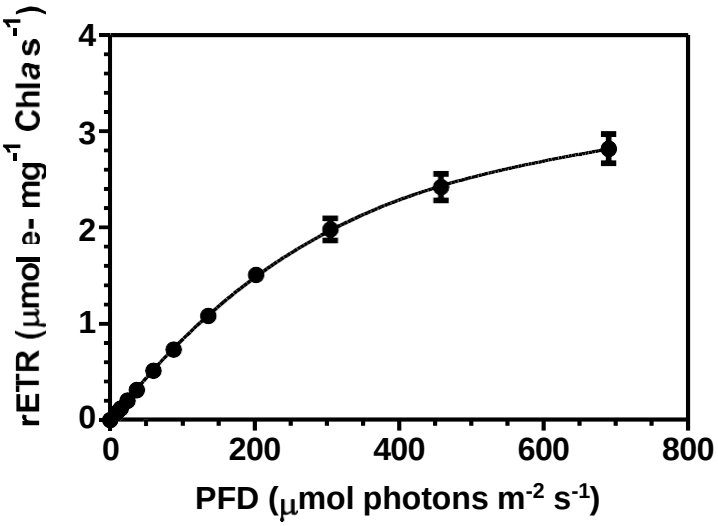


Fig. 9. Relative electron transfer rate (rETR) recorded in cultures of *A. platensis* at the end of the period of exposure to blue light. Data are presented as the mean ± SD of 3 biological replicates.

4. Discussion

The present study shows the effects of different light colors on the growth, photosynthesis and phycocyanin production of *A. platensis* grown under controlled laboratory conditions.

This cyanobacterium absorbs blue light much less efficiently than white and orange light. These differences translated in a better growth under orange and white lights, while under blue light the growth was much lower. These findings are consistent with other

investigations conducted with *A. platensis* [14,16, 20,21] although they used red light instead of orange. Our results support the hypothesis that blue light creates an unbalance between the two photosystems, with an excess of energy capture at the PSI side and a deficiency at the PSII side of the photosynthetic electron transport chain of *A. platensis* [29]. Cyanobacteria invest much more of their chlorophyll *a* in PSI than in PSII [30]. This unbalance between the two photosystems is compensated for by the light-harvesting phycobilisomes (PBSs) which are tendentially associated to PSII [31]. In this way cyanobacteria maintain a balanced excitation distribution between the two photosystems enabling the production of both ATP and NADPH necessary for growth. However, under blue light, the PBSs do not absorb blue photons efficiently because their short wavelength of 450 nm does not match the absorption spectrum of phycocyanin (Fig. 2). Therefore, under blue light the PBSs hardly transfer energy to PSII. On the other hand, chlorophyll *a,* which is more abundant in PSI, can transfer energy to PSI efficiently. Moreover, in cyanobacteria β-carotene, which absorbs blue light efficiently, is more abundant in PSI than in PSII, thus further contributing to photosynthesis light harvesting capacity of PSI. Hence, under blue light the PSII in comparison to PSI experiences a severe limitation of light energy and as result a strong limitation in the linear electron transport. Our results indicate that *A. platensis* cells acclimate to blue light by enhancing the production of PBSs normally serving the PSII, in an attempt to restore the balance between the two photosystems. However, this strategy is ineffective towards growth since PBSs do not absorb blue light. These facts strongly support the findings that the culture grown under blue light have a much lower growth rate compared to orange and white lights, but a significantly higher phycocyanin content.

Metabolically, the favorable effects of blue light on protein synthesis have been extensively investigated for unicellular algae and higher plants, with orange light promoting the accumulation of carbohydrates and blue light enhancing the production of protein [32]. These findings apply also to *A. platensis* which showed a significant increase in proteins.

This may be due to the higher increase in PBSs in thylakoids membranes under blue light than under orange light, while with white light no significant changes were observed.

The chlorophyll-to-dry weight ratio during the growth indicated that orange light induces high cell density, and improve photosynthetic capacity reducing the chlorophyll antenna size or the number of light-harvesting complex [33], while blue light promotes the accumulation of nitrogen compounds such as proteins during the cell growth [32]. Therefore, according to de Mooij et al. 2015 [34], the response of cyanobacteria towards light limiting condition (blue light condition) leads to an increase in the size or number of light harvesting complex. On the other hand, under favorable conditions (orange light) the strategy to increase the productivity is related to the antenna size reduction. Notably, in *A. platensis* the protein content is higher under blue light as compared to the orange-light treatment, mostly due to the fact that the photosynthetic products are spent for synthesizing proteins, particularly phycobiliproteins, instead of promoting cell growth. Therefore, the results obtained in this work confirmed the capability of *A. platensis* to produce the highest phycocyanin content under blue light, as a strategy to counteract to the conditions simulating light limitation due to lack of an inefficient absorption of blue light by phycobilisomes which strongly slows down the growth. Interestingly, the mean growth rate of phycocyanin in blue light and orange light were comparable (0.0210 h^{-1} blue, 0.0225 h^{-1} orange), while the growth rate of biomass strongly diverged (0.0183 h^{-1} blue, vs 0.028 h^{-1} orange). This confirms that growth was much more limited than phycocyanin synthesis which led to an accumulation of phycocyanin in the cells under blue light.

At the end of the experiment, the content (% of dry weight) of allophycocyanin was higher under the blue light conditions. This was possible because the allophycocyanin central cores are surrounded by phycocyanin rods on the border of the phycobilisomes, which are attached to the external surface of the thylakoid membrane. These results are consistent with the finding of Lee et al. 2015 [18] and Park et al. 2019 [35], where the

allophycocyanin and phycocyanin contents are higher under blue light compare to the red light using *A. platensis* and *A. maxima* respectively.

Commercialization of many natural products requires, (i) a large amount of biomass, (ii) high percentage of the desired product in the biomass, and (iii) an industrial scale process. In this respect the growth of *A. platensis* under blue light fulfills the requirement of higher concentration of phycocyanin in the biomass. Yet, increased phycocyanin content resulted decoupled from growth compared to orange light, and proceeded at a much lower growth rate. However, the cultures shifted to blue light retained 60-70% of their rETR as compared to that continuously grown under blue light. It is conceivable that the lower activity was due to acclimation to low light conditions created by the high culture density (about 5 g/l). Orange light, is an ideal light source for cyanobacteria growth since it is fully absorbed by phycocyanin which harnesses light to be transferred to PSII reaction center. However, when comparing the total amount of phycocyanin produced within the same time by the cultures, the orange exposed cultures performed much better. Indeed, the total phycocyanin produced under orange light was 1.7 times higher than that produced under blue light. Moreover, if we consider that leftover biomass after the extraction of phycocyanin still contains valuable products such as γ-linoleic acid, chlorophyll *a* and valuable proteins, the use of orange light could result in a more economical viable production process.

A two-step strategy to produce desired fine chemicals is often applied, for example for the production of astaxanthin in *Haematococcus* [36], β-carotene in *Dunaliella* [37], and for induction of lipid synthesis in *Nannochloropsis* [38]. Therefore, it may be useful to produce *Arthrospira* biomass under orange light, and once the culture is close to the stationary phase, to shift the light to blue to further enhance phycocyanin content. It is expected that a similar behavior may occur between white light (growth phase) to blue light (phycocyanin enrichment phase) [39]. In our experiment, after 24 hours of exposure to blue light a clear drop in phycocyanin content was observed. At the time of the shift to blue light, the culture

had reached a stable dry weight of 4.8 g L^{-1}, therefore, assuming a 10% of nitrogen in the biomass, and considering the initial 2.5 g L^{-1} $NaNO_3$ supplied at the start by Zarrouk's medium, nitrogen presence in the culture medium would have been almost exhausted. This may have triggered the partial phycocyanin degradation [8]. Moreover, the exposure to blue light can stimulate protein synthesis [32]. Therefore, the combination of these two factors may account for the reduction of phycocyanin content. The culture subsequently recovered the PC content, which then increased from 9% to 12% of DW. Therefore, it is advisable to use a two-step process to improve the economic feasibility of the process [35]. This strategy can be successful by coupling growth of culture under orange light to accelerate the increase in biomass, and subsequently exposure of the dense cultures to blue light to achieve a further increase in the concentration of PC in the cells.

Although carotenoids have great commercial importance, in literature there are only few studies that investigate their production in *A. platensis* under different light spectra [17,20]. According to Markou et al. 2014 [17], the carotenoid content was unaffected by the various light colors. By contrast, Lima et al. 2018 [20] found that the concentration of total carotenoids per culture volume were higher for the culture illuminated with LEDs spectral composed by 70% red and 30% blue. However, they also observed that by using continuous blue and red lights, the carotenoid content was higher under red light. Our results are in contrast with those obtained from Markou et al 2014 [17] and Lima et al. 2018 [20]. Although there is an acclimation to the light condition after 24h, the carotenoids profiles showed an increase of their contents during the whole duration of the experiment. In particular, the cultures exposed to the blue light showed the highest carotenoid content in terms of % of DW. This finding is consistent with the results observed by Wilson et al. 2006 where the carotenoids act as a photoreceptors and mediators that respond to the blue light and subsequently induce energy dissipation (and fluorescence quenching) through energy transfer from the phycobilisomes to the photosystems [40].

F_v/F_m is an indicator of the photochemical conversion efficiency of PSII in the dark. Higher values of F_v/F_m estimate the higher light utilization efficiency and greater capability to acclimate to low-light conditions. Under blue light conditions, the F_v/F_m value was higher during all the experiment duration, compared to orange and white light cultures. The white light condition mimics the orange during the first three days, and then it was very similar to the blue light condition. This behavior could be explained by the fact that blue light excites mainly the PSI thus promoting the oxidation of the plastoquinone pool and thus an increase of fluorescence.

Under orange light exposure, the electron transfer rate (ETR_{max}) of *A. platensis* remained higher during the experiment than under blue and white light, and this is not surprising since under orange light the growth of cultures was significantly higher, as this radiation is well absorbed by phycocyanin and transferred to PSII. No clear pattern was observed between blue light and white light, although under white light growth was much better than under blue light. It must be pointed out that all the fluorescence measurements were carried out using red light as measuring light and as the actinic one. This may have levelled the differences between white light cells and blue ones, while the orange one fits better with the measuring and actinic light used by Pam-2100. However, previous measurement of photosynthesis carried out in cyanobacteria using blue light showed a clear reduction as compared to white and orange lights [29].

5. Conclusion

From a biotechnological perspective, optimization of the light quality is mandatory to use a light exposure regime that results in maximum conversion of light to biomass and bioactive compounds production. Unfortunately, very often it is difficult to attain high growth rates of biomass and high production of the desired fine chemicals. The exposure to blue light

triggers the production of proteins, phycocyanin and carotenoids, while the orange light promotes the biomass growth. In an attempt to further improve the production of phycocyanin by *A. platensis,* a two-step light exposure process comprising growth under orange light followed by blue light exposure of dense cultures was tested. It was shown that even dense cultures in their stationary phase can still significantly increase their phycocyanin content, which indicated that synthesis of phycocyanin and increase of dry weight can proceed independently. Therefore, we suggest to produce biomass preferably under orange light and then expose the biomass to blue light to increase the phycocyanin content and make the extraction process more economically convenient.

CRediT Author Contributions Statement

G. Chini Zittelli, B. Cicchi, M. Milia: Methodology, Investigation, Editing; G. Mugnai Summary of the results, Writing, Editing. G. Torzillo, J.: Conceptualization, Writing - original draft, Fund acquisition, Supervision. A. Angioni, P. Addis, Fund acquisition, Supervision.

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Conflict of interest statement

There are no conflicts of interest related to this paper and there are no significant financial support to declare.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

6. References

[1] R. MacColl, Cyanobacterial phycobilisomes, J. Struct. Biol. 124 (1998) 311–334. https://doi.org/10.1006/jsbi.1998.4062.

[2] D. Kirilowsky, Modulating energy arriving at photochemical reaction centers: orange carotenoids protein-related photoprotecting and state transitions. Photosynth. Res. 126 (2015) 3-17. 10.1007/s11120-014-0031-7

[3] Q. Liu, Y. Huang, R. Zhang, T. Cai, Y. Cai, Medical Application of Spirulina platensis Derived C-Phycocyanin, Evidence-Based Complement. Altern. Med. 2016 (2016). https://doi.org/10.1155/2016/7803846.

[4] P. Yu, Y. Wu, G. Wang, T. Jia, Y. Zhang, Purification and bioactivities of phycocyanin, Crit. Rev. Food Sci. Nutr. 57 (2017) 3840–3849. https://doi.org/10.1080/10408398.2016.1167668.

[5] Insights, Future Market 2018. Phycocyanin Market: Food & Beverage Application to Hold Close to 85% Value Share Throughout the Forecast Period: Global Industry Analysis (2013 - 2017) & Opportunity Assessment (2018 - 2028). https://www.marketresearch.com/Future-Market-Insights-v4066/Phycocyanin-Food-Beverage-Application-Hold-11865287/

[6] S.K. Ojir, The response of phycobiliproteins to light qualities in *Anabaena circularis*. j. Appl. Biol. Biotechnol., 3 (2015) 1-6. DOI: 10.7324/JABB.2015.3301

[7] R. Lauceri, G. Chini Zittelli, B. Maserti, G. Torzillo, Purification of phycocyanin from *Arthrospira platensis* by hydrophobic interaction membrane chromatography, Algal Rresearch, 35 (2018) 333-340. DOI: 10.1016/j.algal.2018.09.003

[8] S. Boussiba, A. Richmond, C-Phycocyanin as a storage protein in the be green alga *Spirulina platensis,* Arch. Microbiol.125 (1980) 143-147. 0302-8933/80/0125/0143/$01.00

[9] X. Li, W. Li, J, Zhai, X. Way, Effect of nitrogen limitation on biochemical composition and photosynthetic performance for fed-batch mixotrophic cultivation of microalga *Spirulina platensis*, Biotechnol. Biores, 263 (2018) 555-561. DOI: 10.1016/j.biortech.2018.05.046

[10] G. Torzillo, B. Pushparaj, F. Bocci, W. Balloni, R. Materassi, G. Florenzano, Production of *Spirulina* biomass in closed photobioreactors, Biomass 11 (1986) 61–74.

[11] T.S. Babu, A. Kumar, A.K. Varma, Effect of light quality on phycobilisome components of the cyanobacterium *Spirulina platensis*, Plant Physiol. 95 (1991) 492–497. https://doi.org/10.1104/pp.95.2.492.

[12] P.H. Ravelonandro, D.H. Ratianarivo, C. Joannis-Cassan, A. Isambert, M. Raherimandimby, Influence of light quality and intensity in the cultivation of *Spirulina platensis* from Toliara (Madagascar) in a closed system, J. Chem. Technol. Biotechnol. 83 (2008) 842–848. https://doi.org/10.1002/jctb.1878.

[13] H.K. Madhyastha, T.M. Vatsala, Pigment production in *Spirulina fusiformis* in different photophysical conditions, Biomol. Eng. 24 (2007) 301–305. https://doi.org/10.1016/j.bioeng.2007.04.001.

[14] C.Y. Wang, C.C. Fu, Y.C. Liu, Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*, Biochem. Eng. J. 37 (2007) 21–25. https://doi.org/10.1016/j.bej.2007.03.004.

[15] A. Walter, J.C. de Carvalho, V.T. Soccol, A.B.B. de Faria, V. Ghiggi, C.R. Soccol, Study of phycocyanin production from *Spirulina platensis* under different light spectra, Brazilian Arch. Biol. Technol. 54 (2011) 675–682. https://doi.org/10.1590/S1516-

89132011000400005.

[16] H.B. Chen, J.Y. Wu, C.F. Wang, C.C. Fu, C.J. Shieh, C.I. Chen, C.Y. Wang, Y.C. Liu, Modeling on chlorophyll a and phycocyanin production by *Spirulina platensis* under various light-emitting diodes, Biochem. Eng. J. 53 (2010) 52–56. https://doi.org/10.1016/j.bej.2010.09.004.

[17] G. Markou, Effect of various colors of light-emitting diodes (LEDs) on the biomass composition of *Arthrospira platensis* cultivated in semi-continuous mode, Appl. Biochem. Biotechnol. 172 (2014) 2758–2768. https://doi.org/10.1007/s12010-014-0727-3.

[18] S.H. Lee, J.E. Lee, Y. Kim, S.Y. Lee, The Production of High Purity Phycocyanin by *Spirulina platensis* Using Light-Emitting Diodes Based Two-Stage Cultivation, Appl. Biochem. Biotechnol. 178 (2016) 382–395. https://doi.org/10.1007/s12010-015-1879-5.

[19] M.B. Bachchhav, M.V. Kulkarni, A.G. Ingale, Enhanced Phycocyanin Production from *Spirulina platensis* using Light Emitting Diode, J. Inst. Eng. Ser. E. 98 (2017) 41–45. https://doi.org/10.1007/s40034-016-0090-8.

[20] G.M. Lima, P.C.N. Teixeira, C.M.L.L. Teixeira, D. Filócomo, C.L.S. Lage, Influence of spectral light quality on the pigment concentrations and biomass productivity of *Arthrospira platensis,* Algal Res. 31 (2018) 157–166. https://doi.org/10.1016/j.algal.2018.02.012.

[21] H.A. Wicaksono, W.H. Satyantini, E.D. Masithah, The spectrum of light and nutrients required to increase the production of phycocyanin Spirulina platensis, IOP Conf. Ser. Earth Environ. Sci. 236 (2019). https://doi.org/10.1088/1755-1315/236/1/012008.

[22] H. Liu, R.E. Blankenship, On the interface of light-harvesting antenna complexes and reaction centers in oxygenic photosynthesis, Biochim. Biophys. Acta - Bioenerg. 1860 (2019) 148079. https://doi.org/10.1016/j.bbabio.2019.148079.

[23] C. Zarrouk, Contribution a l'etude d'une cyanophycee. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setch. et Gardner) Geitler (Ph.D. thesis), Universite' de Paris, 1966, p. 138.

[24] H.K. Lichtenthaler, Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes, Methods Enzymol. 148 (1987) 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.

[25] A. Bennett, L. Bogorad, Complementary chromatic adaptation in a filamentous blue-green alga, J. Cell Biol. 58 (1973) 419-435. DOI: 10.1083/jcb.58.2.419

[26] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent., J. Biol. Chem. 193 (1951) 265–275.

[27] J. Kromkamp, M. Limbeek, Effect of short-term variation in irradiance on light harvesting and photosynthesis of the marine diatom *Skeletonema costatum*: A laboratory study simulating vertical mixing, J. Gen. Microbiol. 139 (1993) 2277–2284. https://doi.org/10.1099/00221287-139-9-2277.

[28] B. Genty, J.M. Briantais, N.R. Baker, The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, Biochim. Biophys. Acta - Gen. Subj. 990 (1989) 87–92. https://doi.org/10.1016/S0304-4165(89)80016-9.

[29] V.M. Luimstra, J.M. Schuurmans, A.M. Verschoor, K.J. Hellingwerf, J. Huisman, H.C.P. Matthijs, Blue light reduces photosynthetic efficiency of cyanobacteria through an imbalance between photosystems I and II, Photosynth. Res. 138 (2018) 177–189. https://doi.org/10.1007/s11120-018-0561-5.

[30] Y. Fujita, A study on the dynamics features of photosystem stoichiometry: Accomplishments and problems for future studies, Photosynth. Res. 53 (1997) 83 – 93. https://doi.org/10.1023/a:1005870301868.

[31] C.W. Mullineaux, Phycobilisome-reaction centre interaction in cyanobacteria,

Photosynth. Res. 95 (2008) 175–182. https://doi.org/10.1007/s11120-007-9249-y.

[32] F.L. Figueroa, J. Aguilera, F.X. Niell, Red and blue light regulation of growth and photosynthetic metabolism in *Porphyra umbilicalis* (Bangiales, Rhodophyta), Eur. J. Phycol. 30 (1995) 11–18. https://doi.org/10.1080/09670269500650761.

[33] H.M. Amaro, F. Pagels, I.C. Azevedo, J. Azevedo, I. Sousa Pinto, F.X. Malcata, A.C. Guedes, Light-emitting diodes—a plus on microalgae biomass and high-value metabolite production, J. Appl. Phycol. (2020) 3605–3618. https://doi.org/10.1007/s10811-020-02212-2.

[34] T. de Mooij, M. Janssen, O. Cerezo-Chinarro, J.H. Mussgnug, O. Kruse, M. Ballottari, R. Bassi, S. Bujaldon, F.A. Wollman, R.H. Wijffels, Antenna size reduction as a strategy to increase biomass productivity: A great potential not yet realized, J. Appl. Phycol. 27 (2015) 1063–1077. https://doi.org/10.1007/s10811-014-0427-y.

[35] J. Park, T.B. Dinh, Contrasting effects of monochromatic LED lighting on growth, pigments and photosynthesis in the commercially important cyanobacterium *Arthrospira maxima*, Bioresour. Technol. 291 (2019) 121846. https://doi.org/10.1016/j.biortech.2019.121846.

[36] D. Han, Y. Li, Q. Hu, Biology and commercial aspects of *Haematococcus pluvialis* BT - absorption and adsorption of heavy metals by microalgae, Absorpt. Adsorpt. Heavy Met. by Microalgae. (2013) 388–405. http://doi.wiley.com/10.1002/9781118567166.ch20%5Cnpapers3://publication/doi/10.1002/9781118567166.ch20.

[37] M.A. Borowitzka, *Dunaliella* : Biology , Production, and Markets, (2013). In: Richmond, A. and Hu, Q., (eds.) Handbook of Microalgal Culture: Applied Phycology and Biotechnology, 2nd edition. John Wiley & Sons, Ltd, Chichester, West Sussex, UK, pp. 359-368.

[38] L. Rodolfi, G.C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, M.R. Tredici,

Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, Biotechnol. Bioeng. 102 (2009) 100–112. https://doi.org/10.1002/bit.22033.

[39] D.A. García-López, E.J. Olguín, R.E. González-Portela, G. Sánchez-Galván, R. De Philippis, R.W. Lovitt, C.A. Llewellyn, C. Fuentes-Grünewald, R. Parra Saldívar, A novel two-phase bioprocess for the production of *Arthrospira* (Spirulina) *maxima* LJGR1 at pilot plant scale during different seasons and for phycocyanin induction under controlled conditions, Bioresour. Technol. 298 (2020) 122548. https://doi.org/10.1016/j.biortech.2019.122548.

[40] A. Wilson, G. Ajlani, J.M. Verbavatz, I. Vass, C.A. Kerfeld, D. Kirilovsky, A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria, Plant Cell. 18 (2006) 992–1007. https://doi.org/10.1105/tpc.105.040121.

Figure 1

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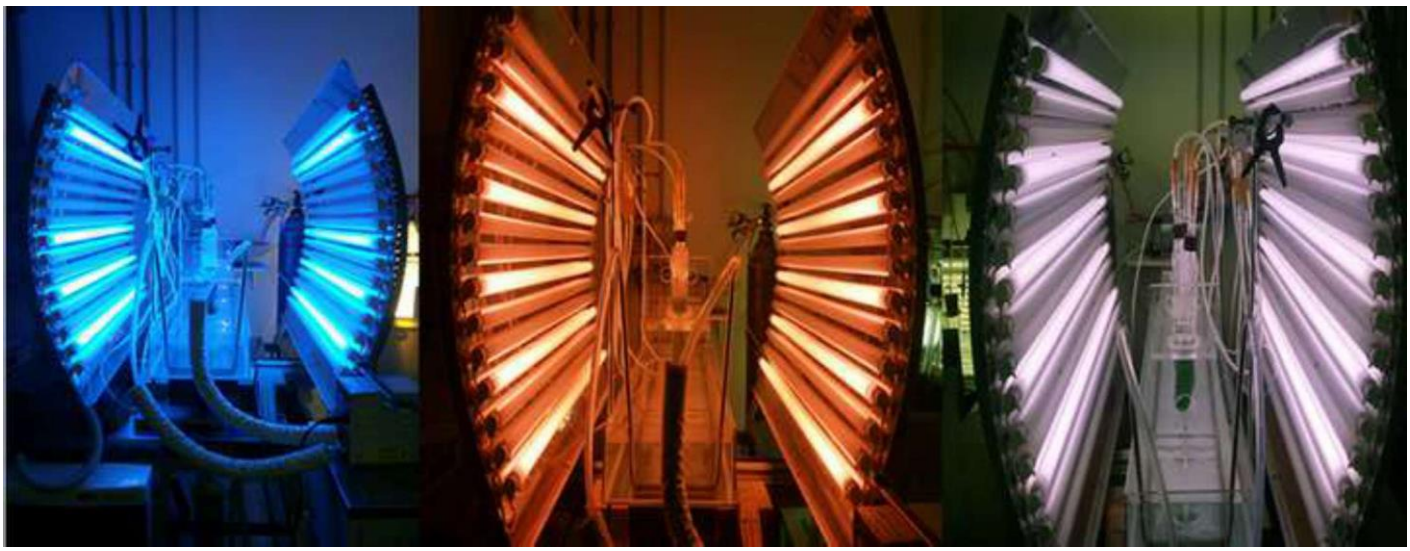


Figure 9

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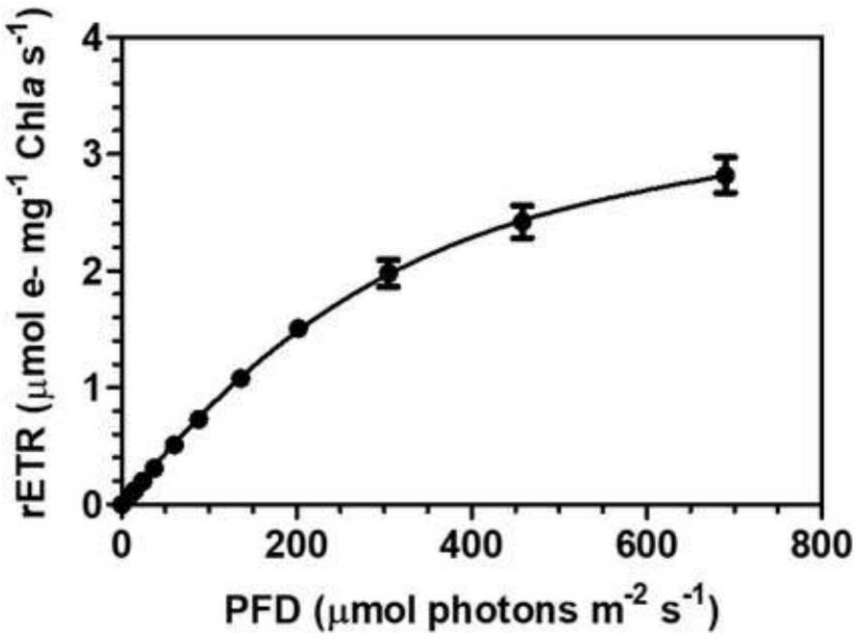


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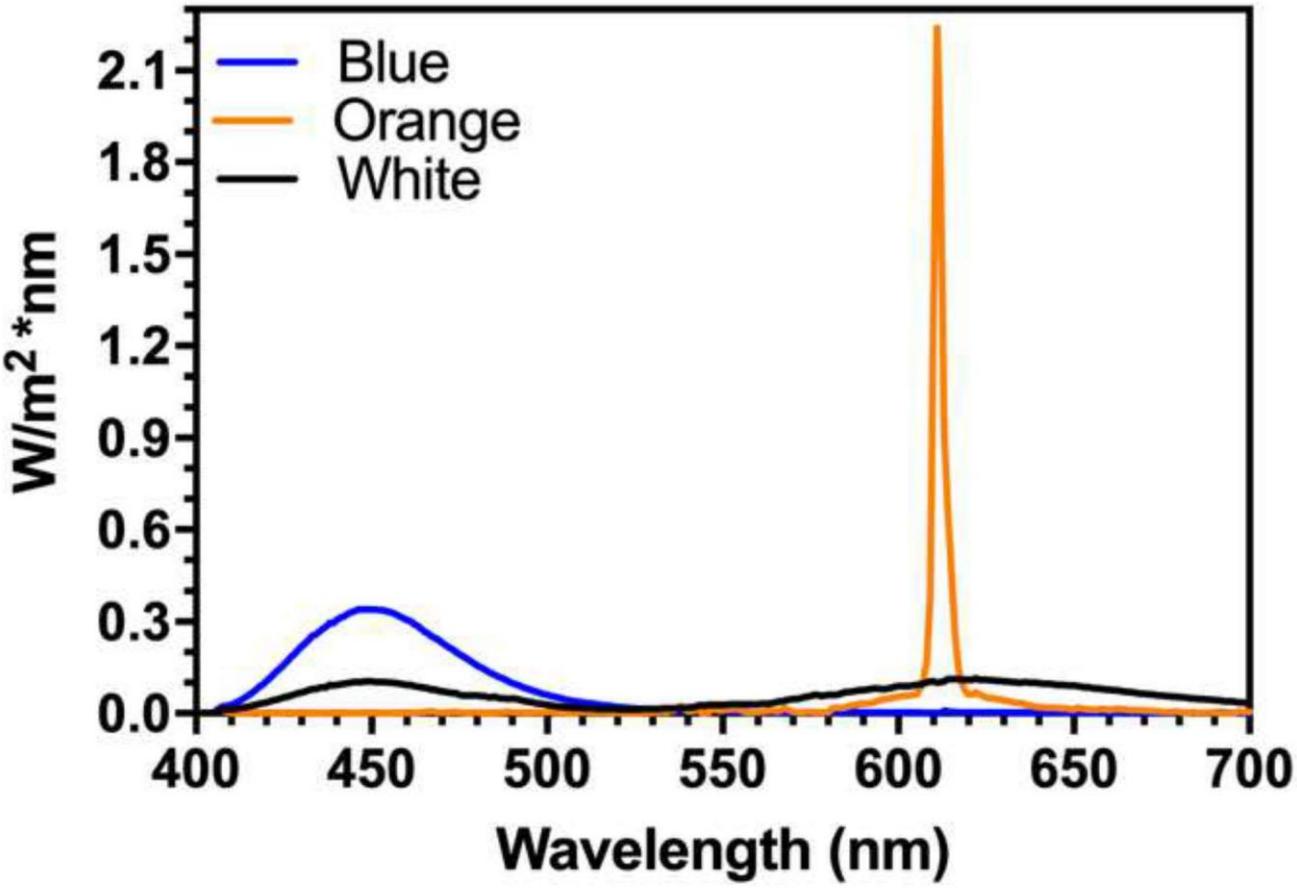


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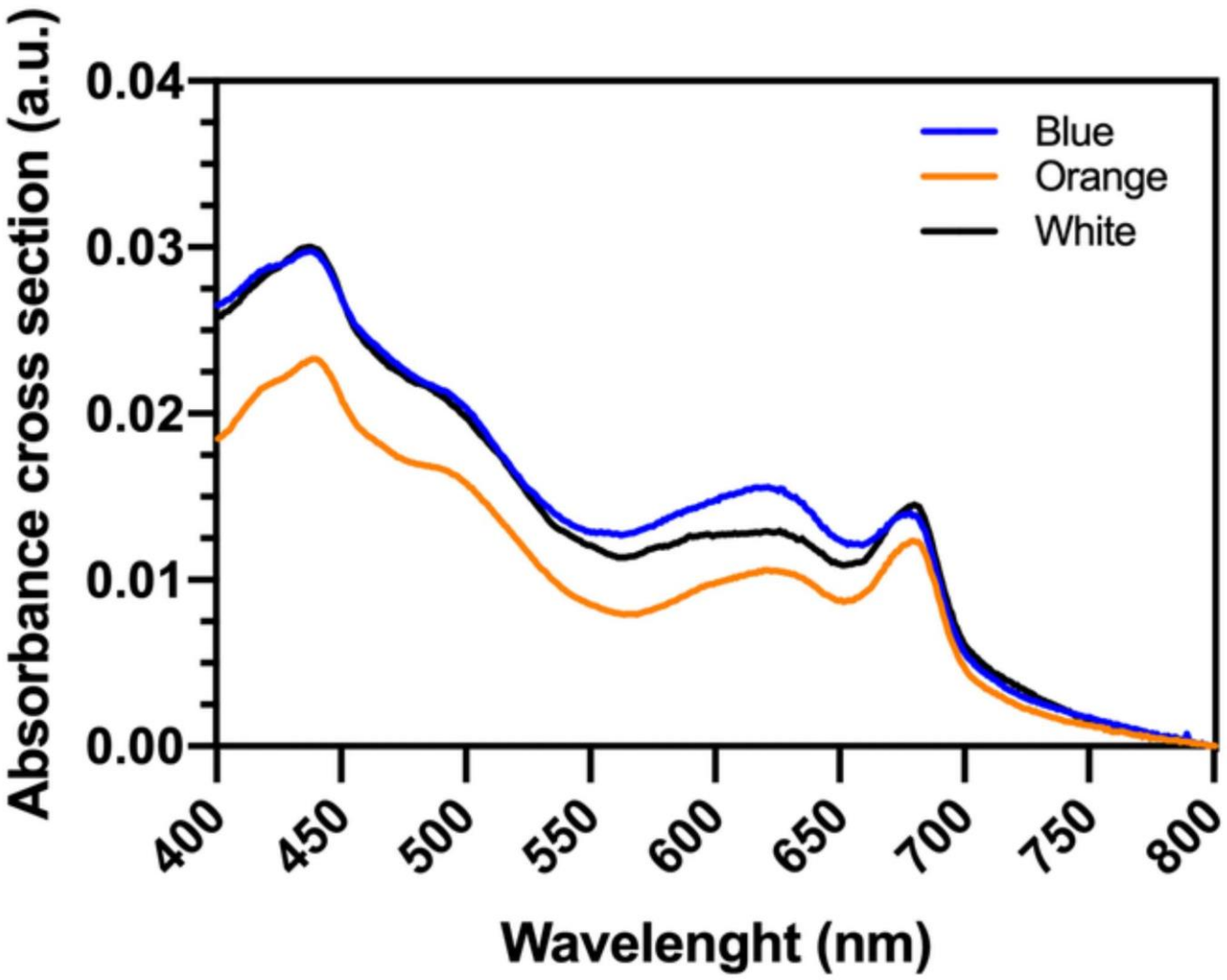


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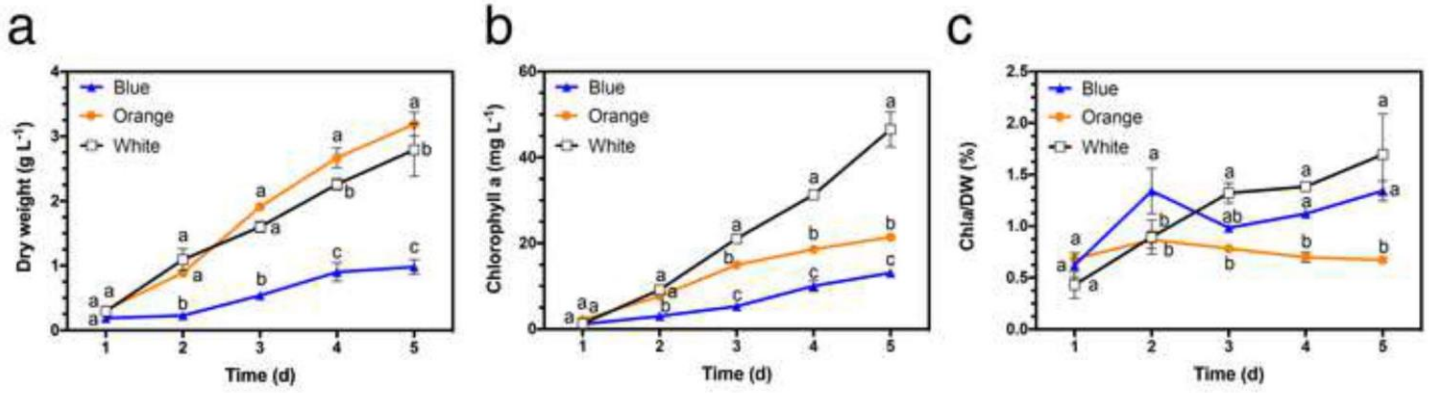


Figure 5

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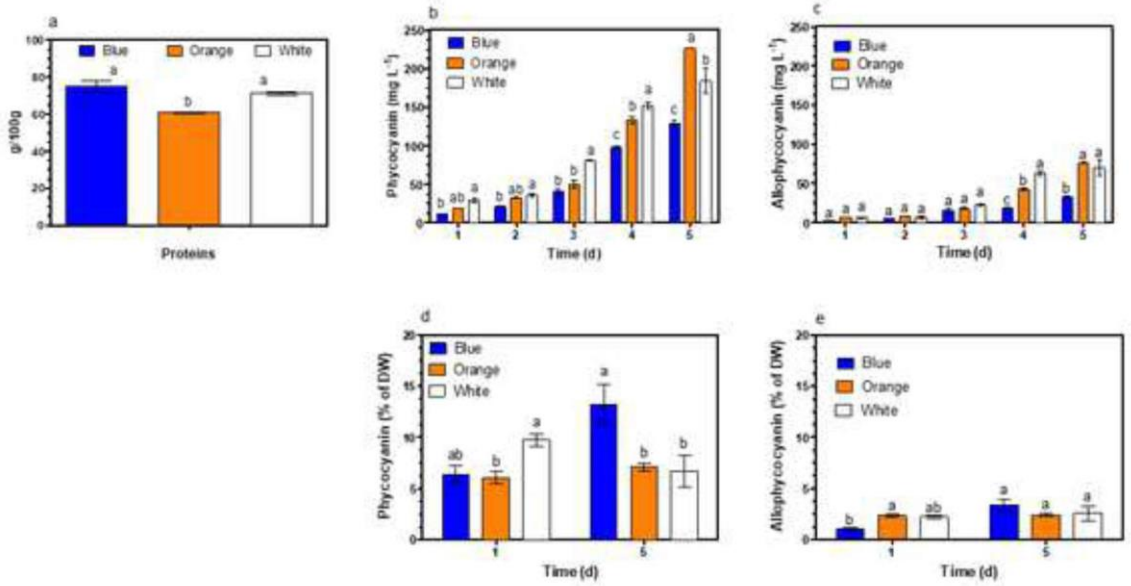


Figure 6

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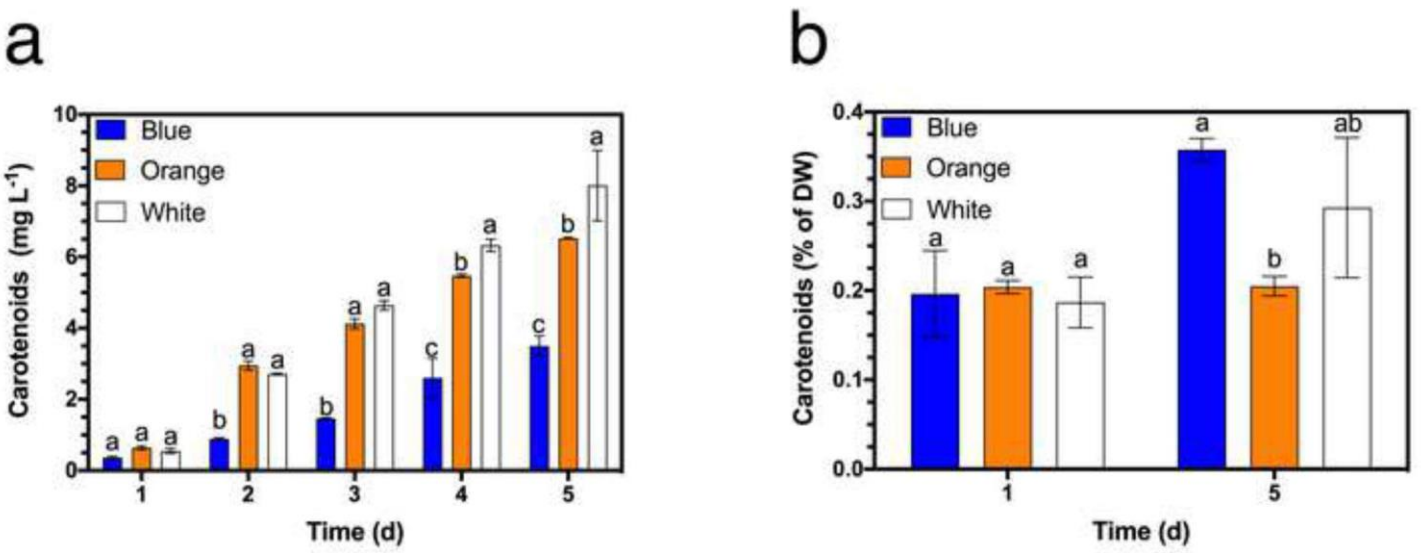


Figure 7

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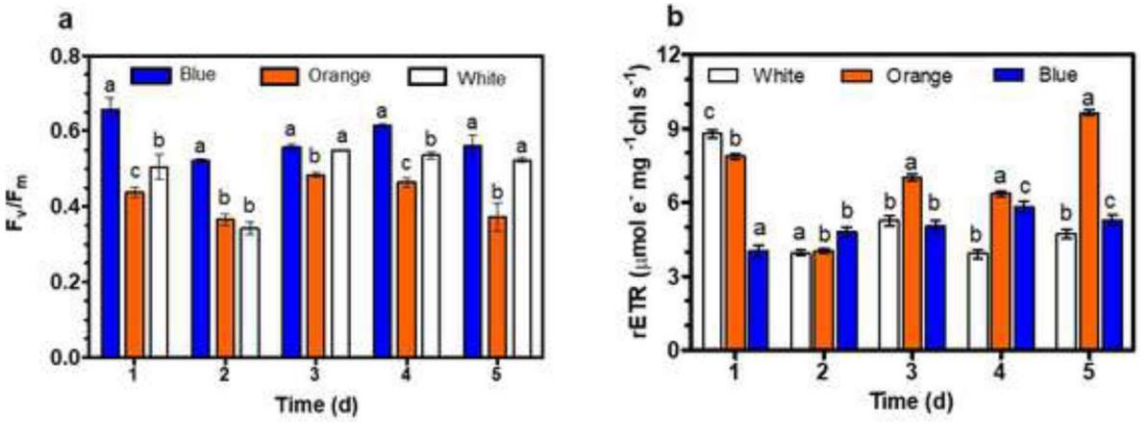
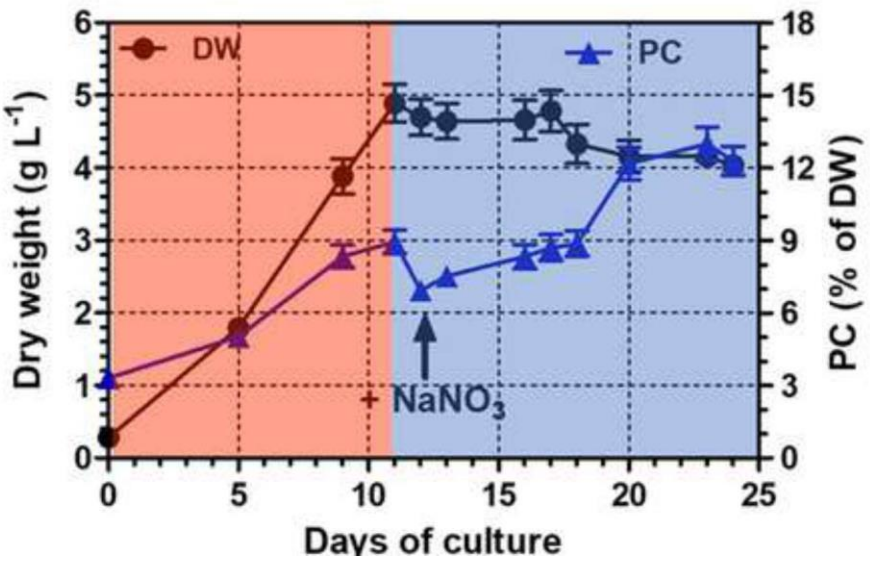


Figure 8

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Conflict of interest statement

There are no conflicts of interest related to this paper and there are no significant financial support to declare.

Author Contributions Statement

G. Chini Zittelli, B. Cicchi, M. Milia: Methodology, Investigation, Editing; G. Mugnai Summary of the results, Writing, Editing. G. Torzillo, J.: Conceptualization, Writing - original draft, Fund acquisition, Supervision. A. Angioni, P. Addis, Fund acquisition, Supervision